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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 5/12, A61K 39/395, C07K 16/00 — A1

(11) International Publication Number: **WO 96/40878**

(43) International Publication Date: 19 December 1996 (19.12.96)

(21) International Application Number: PCT/US96/10053

(22) International Filing Date: 6 June 1996 (06.06.96)

(30) Priority Data:
08/487,550 7 June 1995 (07.06.95) US

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(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: MONKEY MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN B7.1 AND/OR B7.2 PRIMATIZED FORMS, PHARMACEUTICAL COMPOSITIONS

(57) Abstract

The present invention relates to the identification of macaque antibodies to human B7.1 and B7.2 by screening of phase display libraries or monkey heterohybridomas obtained using B lymphocytes from B7.1 and/or B7.2 immunized monkeys. More specifically, the invention provides four monkey monoclonal antibodies 7B6, 16C10, 7C10 and 20C9 which inhibit the B7:CD28 pathway and thereby function as effective immunosuppressants. The invention further provides the complete DNA and amino acid sequences of the light and heavy chain of three primatized antibodies derived from those monkey monoclonal antibodies which bind B7.1 and possibly B7.2, primatized 7C10, primatized 7B6 and primatized 16C10. These primatized and monkey antibodies may be used as specific immunosuppressants, e.g., for the treatment of autoimmune diseases and to prevent organ transplant rejection.

# MONKEY MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN B7.1 AND/OR B7.2 PRIMATIZED FORMS. PHARMACEUTICAL COMPOSITIONS

## FIELD OF THE INVENTION

The present invention relates to the manufacture and identification of novel monoclonal antibodies to human B7, i.e., human B7.1 and human B7.2 and primatized forms thereof. More specifically, the present invention relates to the production and identification of macaque antibodies to human B7, i.e., human B7.1 and human B7.2 produced by screening of phage display libraries and monkey heterohybridomas using B lymphocytes obtained from B7 immunized monkeys.

The invention further relates to specific primatized antibodies which bind to human B7, i.e., human B7.1 and B7.2 as well as their corresponding amino acid and nucleic acid sequences.

Also, the present invention relates to pharmaceutical compositions containing monkey monoclonal or primatized antibodies specific to human B7.1 and/or human B7.2 and their use as immunosuppressants by modulating the B7:CD28 pathway, e.g., for the treatment of autoimmune disorders, and the prevention of organ rejection.

## BACKGROUND OF THE INVENTION

The clinical interface between immunology, hematology, and oncology has long been appreciated. Many conditions treated by the hematologist or oncologist have either an autoimmune or immunodeficient component to their pathophysiology that has led to the widespread adoption of immunosuppressive medications by hematologists, whereas oncologists have sought

M. Lipsky P: "Accessory cell signals involved in T-cell activation." Immunol Rev 117:5, (1990); Weaver CT, Unanue ER: "The co-stimulatory function of antigen-presenting cells." Immunol Today 11:49, (1990); Stennam RM, Young JW: "Signals arising from antigen-presenting cells." Curr Opin Immunol 3:361, (1991)). Co-stimulatory molecules do not initiate but rather enable the generation and amplification of antigen-specific T-cell responses and effector function (Bretscher P: "The two-signal model of lymphocyte activation twenty-one years later." Immunol. Today 13:73, (1992); Jenkins MK, Johnson JG: "Molecules involved in T-cell co-stimulation." Curr Opin Immunol 5:351, (1993); Geppert T, Davis L. Gur H. Wacholtz M. Lipsky P: "Accessory cell signals involved in T-cell activation." Immunol Rev 117:5, (1990); Weaver CT, Unanue ER: "The co-stimulatory function of antigen-presenting cells." Immunol Today 11:49, (1990); Stennam RM, Young JW: "Signals arising from antigen-presenting cells." Curr Opin Immunol 3:361, (1991); June CH, Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28 receptor in T-cell activation." Immunol Today 15:321, (1994).

Recently, one specific co-stimulatory pathway termed B7:CD28 has been studied by different research groups because of its significant role in B and T cell activation (June CH, Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28 receptor in T-cell activation." Immunol Today 15:321, (1994); June CH, Ledbetter JA: "The role of the CD28 receptor during T-cell responses to antigen." Annu Rev Immunol 11:191, (1993); Schwartz RH: "Co-stimulation of T lymphocytes: The role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy." Cell 71:1065, (1992)). Since this

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response to a given antigen (Schwartz, R.H. (1990), "A Cell Culture Model for T Lymphocyte Clonal Anergy." Science, 248:1349; Jenkins, M.K. (1992). "The Role of Cell Division in the Induction of Clonal Anergy." Immunology Today, 13:69; Azuma, M., M. Catabyab, D. Buck, J.H. Phillips, and L.L. Lanier, (1992). "Involvement of CD28 in MHC-unrestricted Cytotoxicity Mediated by a Human Natural Killer Leukemia Cell Line." The Journal of Immunology, 149:1556-1561; Azuma, M., M. Catabyab, D. Buck, J.H. Phillips, and L.L. Lanier, (1992). "CD28 Interaction with B7 Costimulates Primary Allogeneic Proliferative Responses and Cytotoxicity Mediated by Small Resting T Lymphocytes." J. Exp. Med., 175:353-360).

The involvement of certain other co-stimulatory molecules is necessary (Norton, S.D., L. Zuckerman, K.B. Urdahl, R. Shefner, J. Miller, and M.K. Jenkins. (1992), "The CD28 Ligand, B7, Enhances IL-2 Production by Providing A Costimulatory Signal to T Cells." The Journal of Immunology, 149:1556-1561). "The homodimers CD28 and CTLA-4 expressed on T cells" (June, C.H., J.A. Ledbetter, P.S. Linsley, and C.B. Thompson, (1990), "Role of the CD28 Receptor in T-Cell Activation." Immunology Today, 11:211-216; Linsley, P.S., W. Brady, M. Urnes, L.S. Grosmaire, N.K. Damle, and J.A. Ledbetter, (1991), "CTLA-4 is a Second Receptor for the B Cell Activation Antigen B7." J. Exp. Med., 174:561), together with B7.1 (CD80) and B7.2 (CD86) expressed on antigen presenting cells, are major pairs of co-stimulatory molecules necessary for a sustained immune response (Azuma, M., H. Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes." J. Exp. Med., 177:845-850; Freeman, G.J., A.S. Freedman, J.M. Segil,

M.G. Gibson, P.S. Linsley, and J.A. Bluestone, (1992), "Long-Term Survival of Xenogeneic Pancreatic Islet Grafts Induced by CTLA-4Ig." Science, 257:789-795).

The molecules B7.1 and B7.2 can bind to either CD28 or CTLA-4, although B7.1 binds to CD28 with a Kd of 200 Nm and to CTLA-4 with a 20-fold higher affinity (Linsley, P.S., E.A. Clark, and J.A. Ledbetter, (1990), "T-Cell Antigen CD28 Mediates Adhesion with B Cells by Interacting with Activation Antigen B7/BB-1." Proc. Natl. Acad. Sci., 87:5031-5035; Linsley et al, (1993), "The Role of the CD28 receptor during T cell responses to antigen," Annu. Rev. Immunol., 11:191-192; Linesley et al, (1993), "CD28 Engagement by B7/BB-1 Induces Transient Down-Regulation of CD28 Synthesis and Prolonged Unresponsiveness to CD28 Signaling," The Journal of Immunology, 150:3151-3169). B7.2 is expressed on activated B cells and interferon induced monocytes, but not resting B cells (Freeman, G.J., G.S. Gray, C.D. Gimmi, D.B. Lomarrd, L-J. Zhou, M. White, J.D. Fingeroth, J.G. Gribben, and LM. Nadler, (1991). "Structure, Expression and T Cell Costimulatory Activity of the Murine Homologue of the Human B Lymphocyte Activation Antigen B7," J. Exp. Med., 174:625-631). B7.2, on the other hand, is constitutively expressed at very low levels on resting monocytes, dendritic cells and B cells, and its expression is enhanced on activated T cells, NK cells and B lymphocytes (Azuma, M. D. Ito, H. Yagita, K. Okumura, J.H. Phillips, L.L. Lanier, and C. Somoza, "1993", "B70 Antigen is a Second Ligand for CTLA-4 and CD28," Nature, 366:76-79). Although B7.1 and B7.2 can be expressed on the same cell type, their expression on B cells occurs with different kinetics (Lenschow, D.J., G.H. Su, L.A. Zuckerman, N. Nabavi, C.L. Jellis, G.S. Gray, J. Miller, and J.A. Bluestone,

and CD28 on the T cell are currently unknown (Janeway, C.A., Jr. and K. Bottomly, (1994), "Signals and Signs for Lymphocyte Responses," Cell, 76.275285). However, it is possible that one set of receptors could provide the initial stimulus for T cell activation and the second, a sustained signal to allow further elaboration of the pathway and clonal expansion to take place (Linsley, P.S., J.L. Greene, P. Tan, J. Bradshaw, J.A. Ledbetter, C. Anasetti, and N.K. Damle, (1992), "Coexpression and Functional Cooperation of CTLA-4 and CD28 on Activated T Lymphocytes," J. Exp. Med., 176:1595-1604). The current data supports the two-signal hypothesis proposed by Jenkins and Schwartz (Schwartz, R.H., (1990), "A Cell Culture Model for T Lymphocyte Clonal Anergy," Science, 248:1349; Jenkins, M.K., (1992), "The Role of Cell Division in the Induction of Clonal Anergy," Immunology Today, 13:69) that both a TCR and co-stimulatory signal are necessary for T cell expansion, lymphokine secretion and the full development of effector function (Greenan, V. and G. Kroemer, (1993), "Multiple Ways to Cellular Immune Tolerance," Immunology Today, 14:573). The failure to deliver the second signal results in the inability of T cells to secrete IL-2 and renders the cell unresponsive to antigen.

Structurally, both B7.1 and B7.2 contain extracellular immunoglobulin superfamily V and C-like domains, a hydrophobic transmembrane region and a cytoplasmic tail (Freeman, G.J., J.G. Gribben, V.A. Boussiotis, J.W. Ng, V. Restivo, Jr., L.A. Lombard, G.S. Gray, and L.M. Nadler, (1993), "Cloning of B7-2: A CTLA-4 Counter-receptor that Co-stimulates Human T Cell Proliferation," Science, 262:909). Both B7.1 and B7.2 are heavily glycosylated. B7.1 is a 44-54kD

A.S. Freedman, J.M. Segil, G. Lee, J.F. Whitman, and LM. Nadler, (1989), "B7, A New Member of the Ig Superfamily with Unique Expression on Activated and Neoplastic B Cells," The Journal of Immunology, 143:2714-2722). Although alignment of human B7.1 human B7.2 and murine B.1 sequences shows few stretches of lengthy homology, it is known that all three molecules bind to human CTLA-4 and CD28. Thus, there is most likely a common, or closely homologous region shared by the three molecules that may be either contiguous or conformational. This region may constitute the binding site of the B7.1 and B7.2 molecules to their counter-receptors. Antibodies raised against these epitopes could potentially inhibit the interaction of B7 with its counter-receptor on the T cell. Furthermore, antibodies that cross-reacted with this region on both B7.1 and B7.2 molecules would potentially have practical advantages over antibodies directed against B7.1 or B7.2 separately.

**2. Blockade of the B7/CD28 Interaction**

Blocking of the B7/CD28 interaction offers the possibility of inducing specific immunosuppression, with potential for generating long lasting antigen-specific therapeutic effects. Antibodies to either B7.1 or B7.2 have been shown to block T cell activation, as measured by the inhibition of IL-2 production *in vitro* (DeBoer, M., P. Parren, J. Dove, F. Ossendorp, G. van der Horst, and J. Reeder, (1992), "Functional Characterization of a Novel Anti-B7 Monoclonal Antibody," Eur. Journal of Immunology, 22:3071-3075; Azuma, M., H. Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes," J. Exp. Med., 177:845-850). However, different antibodies have been shown to vary in their immunosuppressive

### 3. Recombinant Phage Display Technology for Antibody Selection

To date, no monoclonal antibodies which crossreact with both B7.1 and B7.2 have been reported. As noted, such antibodies would potentially be highly desirable as immunosuppressants. Phage display technology is beginning to replace traditional methods for isolating antibodies generated during the immune response, because a much greater percentage of the immune repertoire can be assessed than is possible using traditional methods. This is in part due to PEG fusion inefficiency, chromosomal instability, and the large amount of tissue culture and screening associated with heterohybridoma production. Phage display technology, by contrast, relies on molecular techniques for potentially capturing the entire repertoire of immunoglobulin genes associated with the response to a given antigen.

This technique is described by Barber et al, Proc. Natl. Acad. Sci., USA, 88, 7978-7982, (1991). Essentially, immunoglobulin heavy chain genes are PCR amplified and cloned into a vector containing the gene encoding the minor coat protein of the filamentous phage M13 in such a way that a heavy chain fusion protein is created. The heavy chain fusion protein is incorporated into the M13 phage particle together with the light chain genes as it assembles. Each recombinant phage contains, within its genome, the genes for a different antibody Fab molecule which it displays on its surface. Within these libraries, in excess of $10^6$ different antibodies can be cloned and displayed. The phage library is panned on antigen coated microliter wells, non-specific phage are washed off, and antigen binding phage are eluted. The genome from the antigen-specific

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cynomolgus monkeys with human antigens or receptors. This technique was developed to create high affinity monoclonal antibodies directed to human cell surface antigens.

Antibodies generated in this manner have previously been reported to display human effector function, have reduced immunogenicity, and long serum half-life. The technology relies on the fact that despite the fact that cynomolgus monkeys are phylogenetically similar to humans, they still recognize many human proteins as foreign and therefore mount an immune response. Moreover, because the cynomolgus monkeys are phylogenetically close to humans, the antibodies generated in these monkeys have been discovered to have a high degree of amino acid homology to those produced in humans. Indeed, after sequencing macaque immunoglobulin light and heavy chain variable region genes, it was found that the sequence of each gene family was 85-98% homologous to its human counterpart (Newman et al, (1992), Id.). The first antibody generated in this way, an anti-CD4 antibody, was 91-92% homologous to the consensus sequence of human immunoglobulin framework regions. Newman et al, Biotechnology, 10:1458-1460, (1992).

Monoclonal antibodies specific to the human B7 antigen have been previously described in the literature. For example, Weyl et al, Hum. Immunol., 31(4), 271-276, (1991) describe epitope mapping of human monoclonal antibodies against HLA-B-27 using natural and mutated antigenic variants. Also, Toubert et al, Clin. Exp. Immunol., 82(1), 16-20, (1990) describe epitope mapping of an HLA-B27 monoclonal antibody that also reacts with a 35-KD bacterial outer membrane protein. Also, Valle et al, Immunol., 69(4), 531-535, (1990)

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libraries and/or monkey heterohybridomas using B lymphocytes obtained from human B7 antigen, i.e., human B7.1 or B7.2 antigen immunized monkeys.

It is another specific object of the invention to provide anti-B7 monkey monoclonal antibodies and primatized forms thereof which specifically bind human B7.1 and/or B7.2 antigen which inhibit the B7/CD86 pathway and B7 stimulation of activated T cells, thereby inhibiting IL-2 production and T cell proliferation and functioning as effective immunosuppressants.

It is another object of the invention to provide anti-human B7.1 and anti-human B7.2 monkey monoclonal antibodies and primatized forms thereof which inhibit antigen driven responses in donor spleen cell cultures, e.g., antigen specific IgG responses, IL-2 production and cell proliferation.

It is another specific object of the invention to identify particular monkey monoclonal antibodies specific to human B7.1 and human B7.2 antigen and primatized forms thereof having advantageous properties, i.e., affinity, immunosuppressive activity, which are useful as therapeutics. More specifically, these monkey antibodies and primatized forms thereof are to be used, e.g., as immunosuppressants, i.e., to block antigen driven immune responses, to treat autoimmune diseases such as psoriasis, rheumatoid arthritis, systemic erythematosus (SLE), type 1 diabetes mellitus, idiopathic thrombocytopenia purpura (ITP), and to prevent organ rejection.

It is another object of the invention to provide pharmaceutical compositions containing one or more monkey monoclonal antibodies specific to human B7 antigen, i.e., human B7.1 and/or human B7.2 antigen, or primatized forms thereof, and a pharmaceutically

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Figure 7 depicts inhibition of IL-2 protein in mixed lymphocyte cultures by anti-B7.1 affinity-purified monkey serum antibodies.

Figure 8a depicts the amino acid and nucleic acid sequence of a primatized form of the light chain of 7C10.

Figure 8b depicts the amino acid and nucleic acid sequence of a primatized form of the heavy chain of 7C10.

Figure 9a depicts the amino acid and nucleic acid sequence of a primatized form of the light chain of 7B6.

Figure 9b depicts the amino acid and nucleic acid sequence of a primatized form of the heavy chain of 7B6.

Figure 10a depicts the amino acid and nucleic acid sequence of a primatized light chain 16C10.

Figure 10b depicts the amino acid and nucleic acid sequence of a primatized heavy chain 16C10.

## DETAILED DESCRIPTION OF THE INVENTION

As described above, the present invention relates to the manufacture of novel monkey monoclonal antibodies which specifically bind human B7.1 and/or human B7.2 antigen, as well as primatized antibodies derived therefrom. These antibodies possess high affinity to human B7.1 and/or B7.2 and therefore may be used as immunosuppressants which inhibit the B7:CD86 pathway.

Preparation of monkey monoclonal antibodies will preferably be effected by screening of phage display libraries or by preparation of monkey heterohybridomas using B lymphocytes obtained from B7 (e.g., human B7.1 and/or B7.2) immunized monkeys.

As noted, the first method for generating anti-B7 antibodies involves recombinant phage display

## Definitions

The following terms are defined so that the invention may be more clearly understood.

Depleting antibody - an antibody which kills activated B cells or other antigen presenting cells.

Non-depleting antibody - an antibody which blocks the co-stimulatory action of B7 and T cell activating ligands CD28 and CTLA-4. Thus, it anergizes but does not eliminate the antigen presenting cell.

Primatized antibody - a recombinant antibody which has been engineered to contain the variable heavy and light domains of a monkey antibody, in particular, a cynomolgus monkey antibody, and which contains human constant domain sequences, preferably the human immunoglobulin gamma 1 or gamma 4 constant domain (or PE variant). The preparation of such antibodies is described in Newman et al, (1992), "Primatization of Recombinant Antibodies for Immunotherapy of Human Diseases: A Macaque/Human Chimeric Antibody Against Human CDH, Biotechnology, 10:1458-1460; also in commonly assigned 08/379,072 both of which are incorporated by reference in their entirety herein. These antibodies have been reported to exhibit a high degree of homology to human antibodies, i.e., 85-98%, display human effector functions, have reduced immunogenicity, and may exhibit high affinity to human antigens.

B7 antigens - B7 antigens in this application include, e.g., human B7, B7.1 and B7.2 antigens. These antigens bind to CD28 and/or CTLA-4. These antigens have a co-stimulatory role in T cell activation. Also, these B7 antigens all contain extracellular immunoglobulin superfamily V and C-like domains, a hydrophobic transmembrane region and a cytoplasmic tail. (See,

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The significant advantage of antibodies obtained from cynomolgus monkeys is that these monkeys recognize many human proteins as foreign and thereby provide for the formation of antibodies, some with high affinity to desired human antigens, e.g., human surface proteins and cell receptors. Moreover, because they are phylogenetically close to humans, the resultant antibodies exhibit a high degree of amino acid homology to those produced in humans. As noted above, after sequencing macaque immunoglobulin light and heavy variable region genes, it was found that the sequence of each gene family was 85-88% homologous to its human counterpart (Newman et al, (1992), Id.).

Essentially, cynomolgus macaque monkeys are administered human B7 antigen, e.g., human B7.1 and/or human B7.2 antigen, B cells are isolated therefrom, e.g., lymph node biopsies are taken from the animals, and B lymphocytes are then fused with KH6/B5 (mouse x human) heteromyeloma cells using polyethylene glycol (PEG). Heterohybridomas secreting antibodies which bind human B7 antigen, e.g., human B7.1 and/or human B7.2 antigen, are then identified.

Antibodies which bind to both B7.1 and B7.2 are desirable because such antibodies potentially may be used to inhibit the interaction of B7.1 and B7.2, as well as B7 with their counter-receptors, i.e., human CTLA-4 and CD28. Antibodies against these epitopes may inhibit the interaction of both human B7.1 and human B7.2 with their counter receptors on the T cell. This may potentially provide synergistic effects.

However, antibodies which bind to only one of human B7 antigen, B7.1 antigen or B7.2 antigen, are also highly desirable because of the co-involvement of these molecules in T cell activation, clonal expansion

human B7, B7.1 or B7.2 antigen will be administered in combination with an adjuvant, e.g., Complete Freund's Adjuvant (CFA), Alum, Saponin, or other known adjuvants, as well as combinations thereof. In general, this will require repeated immunization, e.g., by repeated injection, over several months. For example, administration of soluble B7.1 antigen was effected in adjuvant, with booster immunizations, over a 3 to 4 month period, with resultant production of serum containing antibodies which bound human B7.1 antigen.

After immunization B cells are collected, e.g., by lymph node biopsies taken from the immunized animals and B lymphocytes fused with KH6/B5 (mouse x human) heteromyeloma cells using polyethylene glycol. Methods for preparation of such heteromyelomas are known and may be found in U.S. Serial No. 08/379,072 by Newman et al, filed on January 25, 1995 and incorporated by reference herein.

Heterohybridomas which secrete antibodies which bind human B7, B7.1 and/or B7.2 are then identified. This may be effected by known techniques. For example, this may be determined by ELISA or radioimmunoassay using enzyme or radionuclide labelled human B7, B7.1 and/or B7.2 antigen.

Cell lines which secrete antibodies having the desired specificity to human B7, B7.1 and/or B7.2 antigen are then subcloned to monoclonality.

In the present invention, the inventors screened purified antibodies for their ability to bind to soluble B7.1 antigen coated plates in an ELISA assay, antigen positive B cells, and CHO transfectomas which express human B7.1 antigen on their cell surface. In addition, the antibodies were screened for their ability to block B cell/T cell interactions as measured by IL-2

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Figure 2 and contains the cytomegalovirus promoter/enhancer, the mouse beta globin major promoter, the SV40 origin of replication, the bovine growth hormone polyadenylation sequence, neomycin phosphotransferase exon 1 and exon 2, human immunoglobulin kappa or lambda constant region, the dihydrofolate reductase gene, the human immunoglobulin gamma 1 or gamma 4 PE constant region and leader sequence. This vector has been found to result in very high level expression of primatized antibodies upon incorporation of monkey variable region genes, transfection in CHO cells, followed by selection in G418 containing medium and methotrexate amplification.

For example, this expression system has been previously disclosed to result in primatized antibodies having high avidity (Kd $\leq 10^{-10}$ M) against CD4 and other human cell surface receptors. Moreover, the antibodies have been found to exhibit the same affinity, specificity and functional activity as the original monkey antibody. This vector system is substantially disclosed in commonly assigned U.S. Serial No. 379,072, incorporated by reference herein as well as U.S. Serial No. 08/149,099, filed on November 3, 1993, also incorporated by reference in its entirety herein. This system provides for high expression levels, i.e., > 30 pg/cell/day.

As discussed *infra*, the subject inventors have selected four lead candidate monkey monoclonal antibodies which specifically bind the B7.1 antigen, and which may also bind the B7.2 antigen. These monkey monoclonal antibodies are referred to herein as 7B6, 16C10, 7C10 and 20C9.

As discussed in greater detail *infra*, these antibodies were evaluated for their ability to block B

d: 20C9: $16.8 \times 10^{-9}$M

3. The antibodies were tested *in vitro* in a mixed lymphocyte reaction assay (MLR). The MLR showed that all 4 anti-B7.1 antibodies inhibit IL-2 production to different extents as shown by the following Ibgo values:

a: 7B6: 5.0 μg/M
b: 16C10: <0.1 μg/M
c: 20C9: 2.0 μg/M
d: 7C10: 5.0 μg/M

4. The monkey anti-B7.1 antibodies were tested for their ability to bind B7 on human peripheral blood lymphocytes (PBL). FACS analysis showed that all 4 monkey antibodies tested positive.

5. Monkey antibodies 16C10, 7B6, 7C10 and 20C9 were tested for C1q binding by FACS analysis. Results showed 7C10 monkey Ig had strong human C1q binding after incubating with B7.1 CHO-transfected cells. 16C10 was positive, while 20C9 and 7B6 monkey antibodies were negative.

6. To select an animal model for path-tox studies, the monkey antibodies were tested with animal blood from different species. It was determined that the monkey anti-B7.1 antibodies cross-reacted with human, chimpanzee, and possibly baboon.

Based on these properties, it would appear that three monkey monoclonal antibodies possess the most advantageous properties, 16C10, 7C10 and 20C9, with 16C10 and 7C10 being somewhat better than 20C9.

and IgG4 antibodies are created and tested against specific disease targets.

Given the described binding and functional properties of the subject monkey monoclonal antibodies, these anti-B7.1 monoclonal antibodies and primatized forms thereof should be well suited as therapeutic agents for blocking the B7:CD28 interaction thereby porviding for immunosuppression. In particular, given their high affinity to B7.1 antigen and ability to block B cell/T cell interactions as measured by IL-2 production and tritiated thymidine uptake in mixed lymphocyte culture as well as their ability to effectively inhibit antigen driven responses in donor spleen cell cultures as shown by reduced antigen specific IgG responses, IL-2 production and cell proliferation, these monkey monoclonal antibodies and primatized forms thereof should function as effective immunosuppressants which modulate the B7:CD28 pathway. This is significant for the treatment of many diseases wherein immunosuppression is therapeutically desirable, e.g., autoimmune diseases, to inhibit undesirable antigen specific IgG responses, and also for prevention of organ rejection and graft-versus-host disease. Essentially, the subject antibodies will be useful in treating any disease wherein suppression of the B7:CD28 pathway is therapeutically desirable.

Key therapeutic indications for the subject anti-B7.1 antibodies include, by way of example, autoimmune diseases such as idiopathic thrombocytopenia purpura (ITP), systemic lupus erythematosus (SLE), type 1 diabetes mellitus, multiple sclerosis, aplastic anemia, psoriasis and rheumatoid arthritis.

Another significant therapeutic indication of the subject anti-B7.1 antibodies is for prevention of graft-

techniques well known to those of ordinary skill in the art. The antibodies will generally be provided by standard technique within a pharmaceutically acceptable buffer, and may be administered by any desired route. Because of the efficacy of the presently claimed antibodies and their tolerance by humans it is possible to administer these antibodies repetitively in order to combat various diseases or disease states within a human.

The anti-B7.1 antibodies (or fragments thereof) of this invention are useful for inducing immunosuppression, i.e., inducing a suppression of a human's or animal's immune system. This invention therefore relates to a method of prophylactically or therapeutically inducing immunosuppression in a human or other animal in need thereof by administering an effective, non-toxic amount of such an antibody of this invention to such human or other animal.

The ability of the compounds of this invention to induce immunosuppression has been demonstrated in standard tests used for this purpose, for example, a mixed lymphocyte reaction test or a test measuring inhibition of T-cell proliferation measured by thymidine uptake.

The fact that the antibodies of this invention have utility in inducing immunosuppression indicates that they should be useful in the treatment or prevention of resistance to or rejection of transplanted organs or tissues (e.g., kidney, heart, lung, bone marrow, skin, cornea, etc.); the treatment or prevention of autoimmune, inflammatory, proliferative and hyperproliferative diseases, and of cutaneous manifestations of immunologically medicated diseases (e.g., rheumatoid arthritis, lupus erythematosus,

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expected to be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

The antibodies of the invention may be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce such effect to a therapeutic or prophylactic degree. Such antibodies of the invention can be administered to such human or other animal in a conventional dosage form prepared by combining the antibody of the invention with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

The route of administration of the antibody (or fragment thereof) of the invention may be oral, parenteral, by inhalation or topical. The term parenteral as used herein includes intravenous, intraperitoneal, intramuscular, subcutaneous, rectal or vaginal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred.

The daily parenteral and oral dosage regimens for employing compounds of the invention to prophylactically or therapeutically induce immunosuppression, or to therapeutically treat carcinogenic tumors will generally be in the range of about 0.05 to 100, but preferably about 0.5 to 10, milligrams per kilogram body weight per day.

The antibodies of the invention may also be administered by inhalation. By "inhalation" is meant

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w/w but preferably not in excess of 5% w/w and more preferably from 0.1% to 1% w/w of the formulation.

The topical formulations of the present invention, comprise an active ingredient together with one or more acceptable carrier(s) therefor and optionally any other therapeutic ingredients(s). The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 90°-100°C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

moieties include, by way of example, cytokines such as IL-7 and IL-10, CTLA4-Ig, soluble CTLA-4 and anti-CD28 antibodies and fragments thereof.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of an antibody or fragment thereof of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular animal being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of an antibody or fragment thereof of the invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following formulations are, therefore, to be construed as merely illustrative embodiments and not a limitation of the scope of the present invention in any way.

Capsule Composition

A pharmaceutical composition of this invention in the form of a capsule is prepared by filling a standard two-piece hard gelatin capsule with 50 mg. of an antibody or fragment thereof of the invention, in powdered form, 100 mg. of lactose, 32 mg. of talc and 8 mg. of magnesium stearate.

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Topical Lotion Composition

Antibody or fragment thereof of the invention 1.0 g.

Sorbitan Monolaurate 0.6 g.

Polysorbate 20 0.6 g.

Cetostearyl Alcohol 1.2 g.

Glycerin 6.0 g.

Methyl Hydroxybenzoate 0.2 g.

Purified Water B.P. to 100-00 ml. (B.P. = British Pharmacopeia)

The methyl hydroxybenzoate and glycerin are dissolved in 70 ml. of the water at 75°C. The sorbitan monolaurate, polysorbate 20 and cetostearyl alcohol are melted together at 75°C and added to the aqueous solution. The resulting emulsion is homogenized, allowed to cool with continuous stirring and the antibody or fragment thereof of the invention is added as a suspension in the remaining water. The whole suspension is stirred until homogenized.

Eye Drop Composition

Antibody or fragment thereof of the invention 0.5 g.

Methyl Hydroxybenzoate 0.01 g.

Propyl Hydroxybenzoate 0.04 g.

Purified Water B.P. to 100-00 ml.

The methyl and propyl hydroxybenzoates are dissolved in 70 ml. purified water at 75°C and the resulting solution is allowed to cool. The antibody or fragment thereof of the invention is then added, and the solution is sterilized by filtration through a membrane filter (0.022 μm pore size), and packed aseptically into suitable sterile containers.

compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as Ph adjusting and buffering agents, etc. The concentration of the antibody or fragment thereof of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as i5 or 20% by weight, and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 Ml sterile buffered water, and 50 mg. of an antibody or fragment thereof of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain 250 ml. of sterile Ringer's solution, and 150 mg. of an antibody or fragment thereof of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art, and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania, hereby incorporated by reference herein.

The antibodies (or fragments thereof) of the invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins and art-known lyophilization and reconstitution techniques can be employed.

Depending on the intended result, the pharmaceutical composition of the invention can be

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(Barbas et al, Proc. Natl. Acad. Sci., USA 589:10164-10168, 1992). Although the phage display concept used is substantially similar to that described by Barbas, 1991, Id. the technique has been modified by the substitution of a unique vector for monkey libraries to reduce the possibility of recombination and improve stability. This vector, pMS, Figure 1 contains a single lac promoter/operator for efficient transcription and translation of polycistronic heavy and light chain monkey DNA. This vector contains two different leader sequences, the omp A (Movva et al, J. Biol. Chem., 255: 27-29, (1980), for the light chain and the pel B (Lei, J. Bact., 4379-109:4383 (1987) for the heavy chain Fd. Both leader sequences are translated into hydrophobic signal peptides that direct the secretion of the heavy and light chain cloned products into the periplasmic space. In the oxidative environment of the periplasm, the two chains fold and disulfide bonds form to create stable Fab fragments. We derived the backbone of the vector from the phagemid bluescript. (Stratagene, La Jolla, CA). It contains the gene for the enzyme beta-lactamase that confers ampicillin (carbenicillin) resistance to bacteria that harbor pMS DNA. We also derived, from bluescript, the origin of replication of the multicopy plasmid ColE1 and the origin of replication of the filamentous bacteriophage f1. The origin of replication of phage f1 (the so-called intragenic region), signals the initiation of synthesis of single stranded pMS DNA, the initiation of capsid formation and the termination of RNA synthesis by viral enzymes. The replication and assembly of pMS DNA strands into phage particles requires viral proteins that must be provided by a helper phage. We have used helper phage VCSM13 which is particularly suited for

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where anti-tetanus toxoid phage were combined with phage beading an irrelevant antibody at 1:100,000. We performed three rounds of panning by applying 50 μl of the mixed phage to antigen (tetanus toxoid) coated polystyrene wells. Non-adherent phage were washed off and the adherent phage were eluted with acid. The eluted phage were used to infect a fresh aliquot of XL1-Blue bacteria and helper phage was added. After overnight amplification, phage were prepared and again panned on antigen coated plates. After three rounds of panning, we were able to show that we had successfully enriched for the anti-tetanus toxoid phage. The success of this technology also depends on the ability to prepare soluble Fabs for characterization of the final panned product. This was achieved by excising gene III from the pMS DNA using the restriction enzyme Nhe I followed by re-ligation. After the gene III was excised, the Fab was no longer displayed on the phage surface but accumulated in the piroplasmic space. Lysates were prepared from bacteria expressing soluble Fab and tested for antigen specificity using an ELISA. High levels of soluble Fab were detected.

In order to adapt phage display technology for use with macaque libraries, we developed specific primers for PCR amplifying monkey immunoglobulin genes. These were based on macaque sequences we obtained while developing the PRIMATIZED™ antibody technology (*See*, 08/379,072, incorporated by reference herein) and databases containing human sequences. (Kabat et al, (1991), "Sequences of Proteins of Immunological Interest," U.S. Dept. of Health and Human Services, National Institute of Health).

We developed three sets of primers to cover amplification of the macaque repertoire. Our first set

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The B7.1 fusion protein was generated similarly, except that the PCR amplified B7.1 gene was cloned into a NEOSPLA cassette vector containing the human CH2 and CH3 immunoglobulin genes. CHO cells were transformed with the B7.1/Ig NEOSPLA DNA and stable clones secreting B7.1/Ig fusion protein were amplified. In general, the B7.2 and CTLA4 reagents were generated in the same manner, except that for B7.2 the RNA was isolated from human spleen cells that had been stimulated 24 hours with anti-Ig and IL-4, and for the CTLA4 constructs the gene source was PHA activated human T cells.

**Table 1**

| Reagent | Purpose | CHO Expression |
|---|---|---|
| Soluble B7.1 | Immunization, immunoassays | Yes |
| B7.1 Transfectant | Screening, ELISA | Yes |
| B7.1/Ig Fusion Protein | Inhibition studies, panning | Yes |
| B7.2 Transfectant | Screening, ELISA | Yes |
| B7.2/Ig Fusion Protein | Inhibition studies, panning | To be completed |
| CTLA4 Transfectant | Inhibition studies | To be completed |
| CTLA4/Ig | Inhibition studies | To be completed |

The availability of these reagents, together with monoclonal antibodies to B7.1 (L3074) (Becton Dickinson, 1994) and B7.2 (Fun-1 (Engel et al, Blood, 84, 1402-1407, (1994) and purified goat and rabbit antisera, specifically developed to detect monkey Fab fragments, facilitates identification of antibodies having the desired properties.

lymphocytes were cultured for 3-6 days in the presence of PHA stimulator. B7 binding was detected by radio assay using $^{125}$I-radiolabeled soluble B7.1 (SB7.1).

## Example 5

**Direct binding of monkey antibodies to radiolabeled SB7.** $^{125}$I radiolabeled SB7.1 was tested for binding to anti-B7.1 antibodies at 4, 1 and 0.25 μg/ml in solution. The results shown in Table 2 suggest that most of the antibodies produced by monkeys immunized with SB7.1 were capable of binding the affinity-purified $^{125}$I-SB7.1 in a concentration dependent manner. To evaluate the specificity of binding to labeled SB7.1, unlabelled SB7.1 competition experiments were done with antibodies from two animals. Affinity-purified antibodies from monkeys 1133 and 1144 were coated onto microwell plates at 400 ng/well. Affinity-purified unlabeled SB7.1 (500 and 100 ng/well) was used as competitor. The results shown in Figure 4 demonstrated that SB7.1 preparations are effective in inhibiting the $^{125}$I-SB7.1 from binding to the antibodies.

## Table 2

**Binding of SB7-I$^{125}$ to Monkey Antibodies Affinity Purified on a SB7-Sepharose Affinity Column**

| Antibody (μg/ml) | Monkey Numbers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 769 | 908 | 1133 | 1135 | 1137 | 1139 | 1144 | 1146 |
| 4 | 175 | 213 | 9,056 | 12,771 | 4,318 | 226 | 5,781 | 108 |
| 1 | 106 | 142 | 6,569 | 7,940 | 3,401 | 110 | 3,901 | 80 |
| 0.25 | 95 | 104 | 1,803 | 2,673 | 1,219 | 100 | 1,186 | 94 |

activated T cells was achieved with affinity-purified monkey antibodies at concentrations from 200 to 8 μg/ml. Unlabeled SB7.1 and L307.4 MAb used as controls were also effective in inhibiting B7.1-Ig fusion protein cell binding.

## Example 8

**Inhibition of IL-2 production in mixed lymphocyte reactions by monkey anti-B7 antibodies.**

The blocking of CD28/B7 interaction leads to inhibition of IL-2 production by T lymphocytes. In the experiment shown in Figure 7, affinity-purified monkey antibodies from two monkeys immunized with SB7.1 (monkeys 1137 and 1135) and one immunized with B7 positive SB cells (monkey 1146) were evaluated for their abilities to inhibit human T cell activation in mixed lymphocyte reaction (MLR), as measured by inhibition of IL-2 production. The results of this experiment show that affinity-purified anti-B7.1 antibodies from monkeys 1146 and 1137 inhibited IL-2 production when added at concentrations of 50 μg/ml. Monkey 1135 antibodies could not be evaluated at the two highest concentrations due to lack of material, yet gave significant inhibition at lower concentrations. The murine MAb L307.4 was inhibitory at concentrations of 10 μg/ml. Other monkey sera tested at these concentrations were negative (data not shown). These results demonstrate that at least three of the monkeys immunized with both soluble and membrane associated forms of the B7 antigen are producing B7-blocking antibodies with immunosuppressive potential.

strand cDNA is divided into aliquots and PCR amplified using the sets of kappa, lambda, and heavy chain Fd region primers described earlier and either Pfu polymerase (Stratagene, San Diego) or Taq polymerase (Promega, Madison). The heavy chain PCR amplified products are pooled, cut with Xho VSpe I restriction enzymes and cloned into the vector pMS. Subsequently, the light chain PCR products are pooled, cut with Sac I/Xba I restriction enzymes, and cloned to create the recombinant library. XLI-Blue *E. coli* is transformed with the library DNA and super-infected with VCSM13 to produce the phage displaying antibodies. The library is panned four rounds on polystyrene wells coated with B7.1 or B7.2 antigen. Individual phage clones from each round of panning are analyzed. The pMS vector DNA is isolated and the gene III excised. Soluble Fab fragments are generated and tested in ELISA for binding to B7.1 and B7.2.

## Example 11

**Characterization of Phage Fab Fragments**

The monkey phage Fab fragments are characterized for their specificity and the ability to block B7.1-Ig and B7.2-Ig binding to CTLA-4-Ig or CTLA-4 transfected cells. Phage fragments are also characterized for cross-reactivity after first panning for 4 rounds on the B7 species used for immunization in order to select for high affinity fragments. Fab fragments identified from four rounds of panning either on B7.1 or B7.2 antigen coated surfaces are scaled up by infection and grown in 24 hour fermentation cultures of E coli. Fragments are purified by Kodak FLAG binding to a anti-FLAG affinity column. Purified phage Fabs are tested for affinity by an ELISA based direct binding modified Scatchard

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is similar to the established method used for the generation of monkey anti-CD4 antibodies (Newman, 1992(Id.)). Monkeys with high serum titers will have sections of inguinal lymph nodes removed under anesthesia. Lymphocytes are washed from the tissue and fused with KH6/B5 heteromyeloma cells (Carrol et al, J. Immunol. Meth., 89:61-72, (1986)) using polyethylene glycol (PEG). Hybridomas are selected on H.A.T. media and stabilized by repeated subcloning in 96 well plates.

Monkey monoclonal antibodies specific for B7.1 antigen are screened for cross-reactivity to B7.2. Monkey anti-B7 antibodies will be characterized for blocking of B7/CTLA-4 binding using the $^{125}$I-B7-Ig binding assay. Inhibition of MLR by 3H-Thymidine uptake and direct measurement of IL-2 production is used to select three candidates. Two candidates will be brought forward in Phase II studies and expressed in CHO cells while repeating all functional studies. For the purposes of developing an animal model for *in vivo* pharmacology, anti-B7 antibodies will be tested on cells of several animal species. The establishment of an animal model will allow preclinical studies to be carried out for the selected clinical indication.

**Example 14**

As discussed *supra*, using the above heterohybridoma methods, 4 lead monkey anti-B7.1 antibodies have been identified: 16C10, 7B6, 7C10 and 20C9. These antibodies were characterized as follows:

To demonstrate the monkey antibodies' ability to block the physical interaction between CTLA4-Ig, varying concentrations of the monkey anti-B7.1 antibodies and unlabeled CTLA4-Ig were incubated

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showed 7C10 monkey Ig had strong human C1q binding after incubating with B7.1 CHO-transfected cells. 16C10 was negative, as were the 20C9 and 7B6 monkey antibodies.

## Example 15

Using the primatized antibody methodology incorporated by reference to commonly assigned U.S. Serial No. 08/379,072, and using the NEOSPLA vector system shown in Figure 2, the heavy and light variable domains of 7C10, 7B6 and 16C10 were cloned and primatized forms thereof have been synthesized in CHO cells using the NEOSPLA vector system. The amino acid and nucleic acid sequences for the primatized 7C10 light and heavy chain, 7B6 light and heavy chain, and 16C10 light and heavy chain are respectively shown in Figures 8a, 8b, 9a, 9b, 10a and 10b.

It is expected that these primatized antibodies, given their probable low antigenicity and human effector function, will be well suited as therapeutics. In fact, it has recently been shown that primatized 16C10 exhibits human $C1_9$ binding, whereas 16C10 does not.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be embraced by the following claims.

10. The primatized antibody of claim 5 wherein said antibody is derived from 16C10 and has the amino acid sequence set forth in Figures 4a and 4b.

11. The primatized antibody of claim 5 wherein said antibody is encoded by the nucleic acid sequence set forth in Figures 10a and 10b.

12. A transfectoma which expresses a primatized antibody which specifically binds to human B7.1 and/or human B7.2 antigen.

13. The transfectoma of claim 12 which is a CHO cell.

14. The transfectoma of claim 13 wherein said cell expresses a primatized antibody having the amino acid sequence set forth in any one of Figures 8a, 8b, 9a, 9b, 10a and 10b.

15. A pharmaceutical composition suitable for treatment of a disease treatable by inhibition of B7-CD28 binding which comprises an antibody according to any one of claims 1 to 11.

16. A method of treating a disease by inhibition of the B7:CD28 pathway which comprises administering a therapeutically effective amount of at least one antibody according to any one of claims 1 to 11.

17. The method of claim 16 wherein said antibody is 16C10, 7C10, 20C9, 7B6 or a primatized form thereof.

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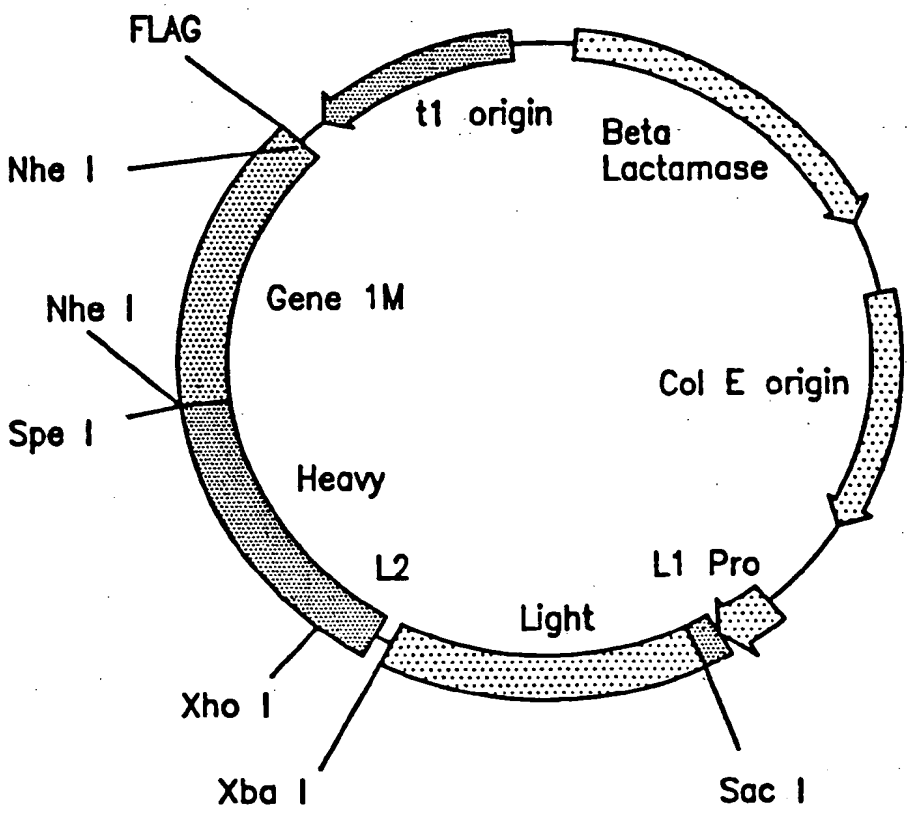
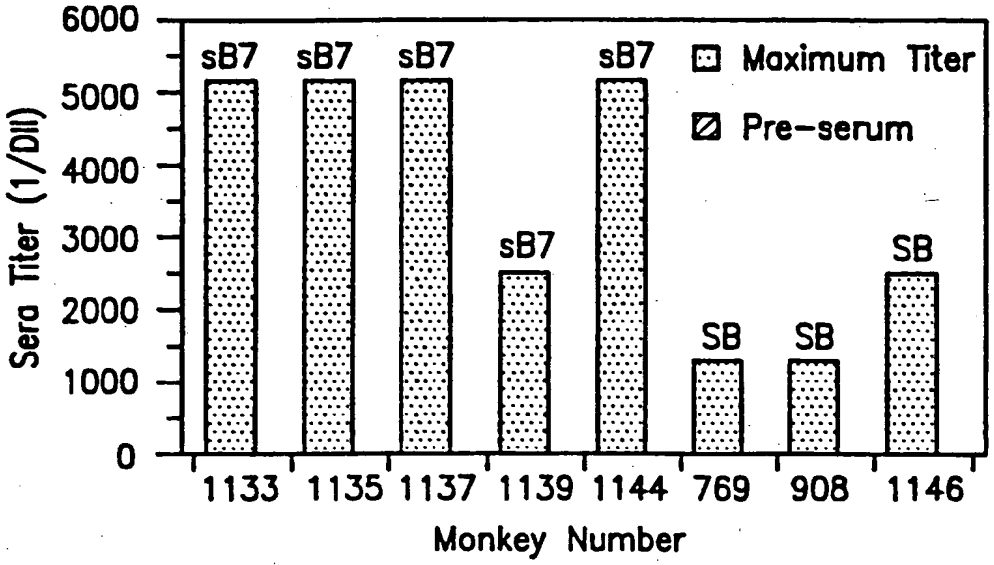

FLAG
Nhe I
Nhe I
Spe I
Xho I
Xba I
t1 origin
Beta Lactamase
Gene 1M
Col E origin
Heavy
L2
L1 Pro
Light
Sac I

FIG. 1

FIG. 3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5000
4000
3000
2000
1000
0
Sera Titer (1/Dil)
sB7
sB7
sB7
sB7
sB7
SB
SB
SB
Maximum Titer
Pre-serum
1133
1135
1137
1139
1144
769
908
1146
Monkey Number

FIG. 4

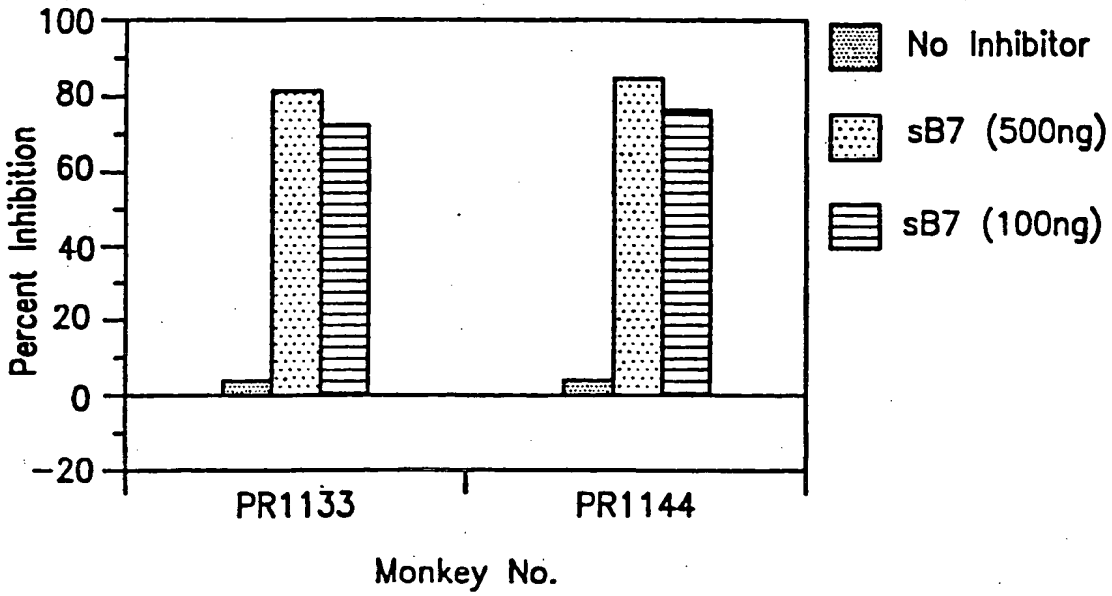
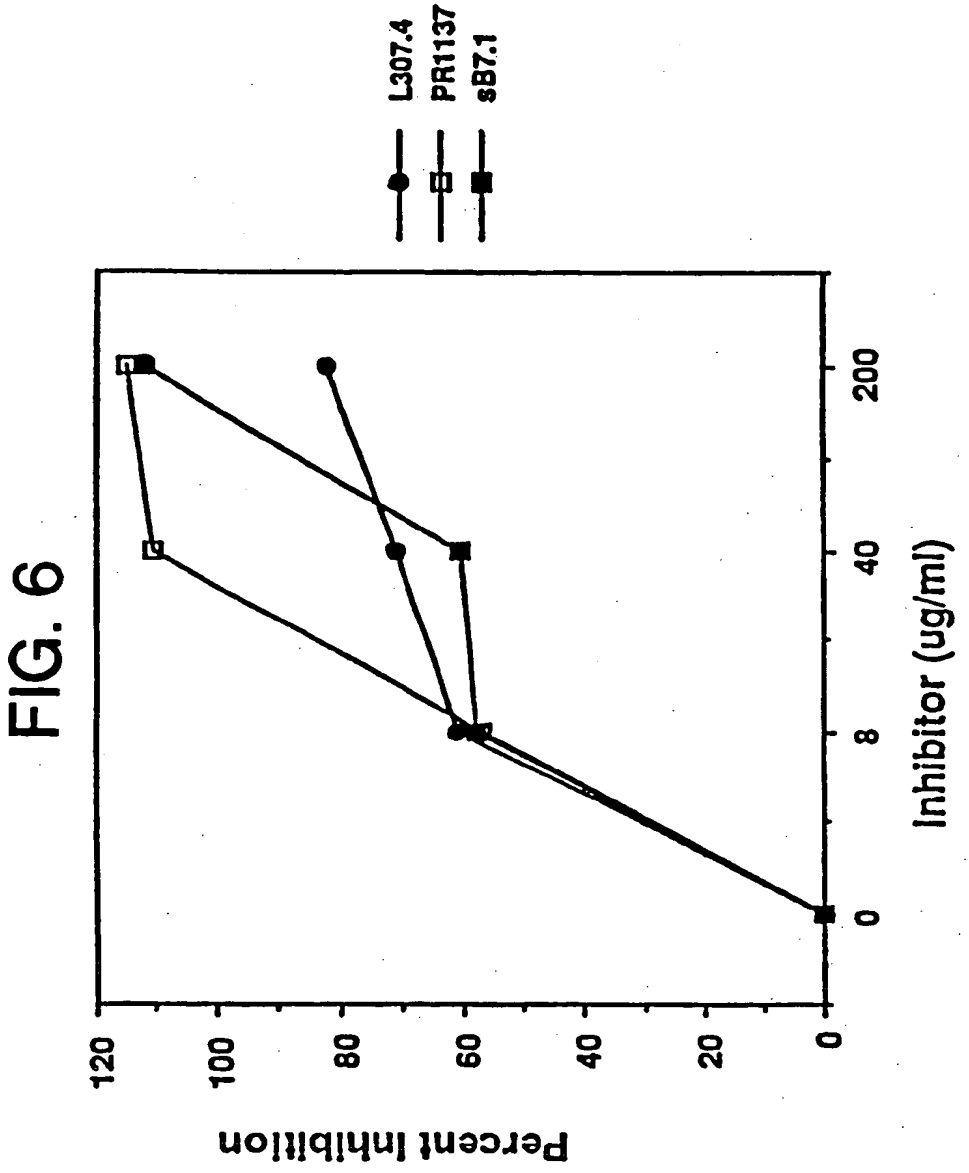

100
80
60
40
20
0
-20
Percent Inhibition
No Inhibitor
sB7 (500ng)
sB7 (100ng)
PR1133
PR1144
Monkey No.

FIG. 6


L307.4
PR1137
sB7.1
Percent Inhibition
120
100
80
60
40
20
0
Inhibitor (ug/ml)
0
8
40
200

```
Frame 1  M   R   V   P   A   Q   L   L   G   L   L   L   L   W   L   P   G   A   R
         ATG AGG GTC CCC GCT CAG CTC CTG GGG CTC CTG CTG CTC TGG CTC CCA GGT GCA CGA
                   9           18          27          36          45          54

         C   A   Y   E   L   T   Q   P   P   S   V   S   V   S   P   G   Q   T   A   R   I
         TGT GCC TAT GAA CTG ACT CAG CCA CCC TCG GTG TCA GTG TCC CCA GGA CAG ACG GCC AGG ATC
               63          72          81          90          99          108         117

         T   C   G   G   D   N   S   R   N   E   Y   V   H   W   Y   Q   Q   K   P   A   R
         ACC TGT GGG GGA GAC AAC AGT AGA AAT GAA TAT GTC CAC TGG TAC CAG CAG AAG CCA GCG CGG
               126         135         144         153         162         171         180

         A   P   I   L   V   I   Y   D   D   S   D   R   P   S   G   I   P   E   R   F   S
         GCC CCT ATA CTG GTC ATC TAT GAT GAT AGT GAC CGG CCC TCA GGG ATC CCT GAG CGA TTC TCT
               189         198         207         216         225         234         243

         G   S   K   S   G   N   T   A   T   L   T   I   N   G   V   E   A   G   D   E   A
         GGC TCC AAA TCA GGG AAC ACC GCC ACC CTG ACC ATC AAC GGG GTC GAG GCC GGG GAT GAG GCT
               252         261         270         279         288         297         306

         D   Y   Y   C   Q   V   W   D   R   A   S   D   H   P   V   F   G   G   G   T   R
         GAC TAT TAC TGT CAG GTG TGG GAC AGG GCT AGT GAT CAT CCG GTC TTC GGA GGA GGG ACC CGG
               315         324         333         342         351         360         369

         V   T   V   L   G   Q   P   K   A   A   P   S   V   T   L   F   P   P   S   S   E
         GTG ACC GTC CTA GGT CAG CCC AAG GCT GCC CCC TCG GTC ACT CTG TTC CCG CCC TCC TCT GAG
               378         387         396         405         414         423         432

         E   L   Q   A   N   K   A   T   L   V   C   L   I   S   D   F   Y   P   G   A   V
         GAG CTT CAA GCC AAC AAG GCC ACA CTG GTG TGT CTC ATA AGT GAC TTC TAC CCG GGA GCC GTG
               441         450         459         468         477         486         495

         T   V   A   W   K   A   D   S   S   P   V   K   A   G   V   E   T   T   T   P   S
         ACA GTG GCC TGG AAG GCA GAT AGC AGC CCC GTC AAG GCG GGA GTG GAG ACC ACC ACA CCC TCC
               504         513         522         531         540         549         558

         K   Q   S   N   N   K   Y   A   A   S   S   Y   L   S   L   T   P   E   Q   W   K
         AAA CAA AGC AAC AAC AAG TAC GCG GCC AGC AGC TAC CTG AGC CTG ACG CCT GAG CAG TGG AAG
               567         576         585         594         603         612         621

         S   H   R   S   Y   S   C   Q   V   T   H   E   G   S   T   V   E   K   T   V   A
         TCC CAC AGA AGC TAC AGC TGC CAG GTC ACG CAT GAA GGG AGC ACC GTG GAG AAG ACA GTG GCC
               630         639         648         657         666         675         684

         P   T   E   C   S
         CCT ACA GAA TGT TCA TGA
               693         702
```

FIG. 8a



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```
H   N   A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S   V
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC
    945         954         963         972         981         990         999

L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   K
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA
    1008        1017        1026        1035        1044        1053        1062

A   L   P   A   P   I   E   K   T   I   S   K   A   K   G   Q   P   R   E   P   Q
GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG
    1071        1080        1089        1098        1107        1116        1125

V   Y   T   L   P   P   S   R   D   E   L   T   K   N   Q   V   S   L   T   C   L
GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG
    1134        1143        1152        1161        1170        1179        1188

V   K   G   F   Y   P   S   D   I   A   V   E   W   E   S   N   G   Q   P   E   N
GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC
    1197        1206        1215        1224        1233        1242        1251

N   Y   K   T   T   P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L
AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC
    1260        1269        1278        1287        1296        1305        1314

T   V   D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E   A
ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT
    1323        1332        1341        1350        1359        1368        1377

L   H   N   H   Y   T   Q   K   S   L   S   L   S   P   G   K
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
    1386        1395        1404        1413        1422        1431
```

FIG. 8b-2

```
Frame 1  M   G   W   S   L   I   L   L   F   L   V   A   V   A   T   R   V   Q   C
         ATG GGT TGG AGC CTC ATC TTG CTC TTC CTT GTC GCT GTT GCT ACG CGT GTC CAG TGT
                   9          18          27          36          45          54

         E   V   Q   L   V   E   S   G   G   G   L   V   Q   P   G   G   S   L   R   V   S
         GAG GTG CAA CTG GTG GAG TCT GGG GGA GGC TTG GTC CAG CCT GGC GGG TCC CTG AGA GTC TCC
               63          72          81          90          99          108         117

         C   A   V   S   G   F   T   F   S   D   H   Y   M   Y   W   F   R   Q   A   P   G
         TGT GCA GTC TCT GGA TTC ACC TTC AGT GAC CAC TAC ATG TAT TGG TTC CGC CAG GCT CCA GGG
               126         135         144         153         162         171         180

         K   G   P   E   W   V   G   F   I   R   N   K   P   N   G   G   T   T   E   Y   A
         AAG GGG CCG GAA TGG GTA GGT TTC ATT AGA AAC AAA CCG AAC GGT GGG ACA ACA GAA TAC GCC
               189         198         207         216         225         234         243

         A   S   V   K   D   R   F   T   I   S   R   D   D   S   K   S   I   A   Y   L   Q
         GCG TCT GTG AAA GAC AGA TTC ACC ATC TCC AGA GAT GAT TCC AAA AGC ATC GCC TAT CTG CAA
               252         261         270         279         288         297         306

         M   S   S   L   K   I   E   D   T   A   V   Y   Y   C   T   T   S   Y   I   S   H
         ATG AGC AGC CTG AAA ATC GAG GAC ACG GCC GTC TAT TAC TGT ACT ACA TCC TAC ATT TCA CAT
               315         324         333         342         351         360         369

         C   R   G   G   V   C   Y   G   G   Y   F   E   F   W   G   Q   G   A   L   V   T
         TGT CGG GGT GGT GTC TGC TAT GGA GGT TAC TTC GAA TTC TGG GGC CAG GGC GCC CTG GTC ACC
               378         387         396         405         414         423         432

         V   S   S   A   S   T   K   G   P   S   V   F   P   L   A   P   S   S   K   S   T
         GTC TCC TCA GCT AGC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC
               441         450         459         468         477         486         495

         S   G   G   T   A   A   L   G   C   L   V   K   D   Y   F   P   E   P   V   T   V
         TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG
               504         513         522         531         540         549         558

         S   W   N   S   G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S
         TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA
               567         576         585         594         603         612         621

         G   L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q   T   Y
         GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG ACC TAC
               630         639         648         657         666         675         684

         I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K   A   E   P   K   S
         ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GCA GAG CCC AAA TCT
               693         702         711         720         729         738         747

         C   D   K   T   H   T   C   P   P   C   P   A   P   E   L   L   G   G   P   S   V
         TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC
               756         765         774         783         792         801         810

         F   L   F   P   P   K   P   K   D   T   L   M   I   S   R   T   P   E   V   T   C
         TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC
               819         828         837         846         855         864         873

         V   V   V   D   V   S   H   E   D   P   E   V   K   F   N   W   Y   V   D   G   V
         GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG
               882         891         900         909         918         927         936
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FIG. 9b-1

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Frame 1  M   R   V   P   A   Q   L   L   G   L   L   L   L   W   L   P   G   A   R
         ATG AGG GTC CCC GCT CAG CTC CTG GGG CTC CTG CTG CTC TGG CTC CCA GGT GCA CGA
                   9                  18                  27                  36                  45                  54

         C   E   S   V   L   T   Q   P   P   S   V   S   G   A   P   G   Q   K   V   T   I
         TGT GAG TCT GTC CTG ACA CAG CCG CCC TCA GTG TCT GGG GCC CCA GGG CAG AAG GTC ACC ATC
               63                  72                  81                  90                  99                 108                 117

         S   C   T   G   S   T   S   N   I   G   G   Y   D   L   H   W   Y   Q   Q   L   P
         TCG TGC ACT GGG AGC ACC TCC AAC ATT GGA GGT TAT GAT CTA CAT TGG TAC CAG CAG CTC CCA
               126                 135                 144                 153                 162                 171                 180

         G   T   A   P   K   L   L   I   Y   D   I   N   K   R   P   S   G   I   S   D   R
         GGA ACG GCC CCC AAA CTC CTC ATC TAT GAC ATT AAC AAG CGA CCC TCA GGA ATT TCT GAC CGA
               189                 198                 207                 216                 225                 234                 243

         F   S   G   S   K   S   G   T   A   A   S   L   A   I   T   G   L   Q   T   E   D
         TTC TCT GGC TCC AAG TCT GGT ACC GCG GCC TCC CTG GCC ATC ACT GGG CTC CAG ACT GAG GAT
               252                 261                 270                 279                 288                 297                 306

         E   A   D   Y   Y   C   Q   S   Y   D   S   S   L   N   A   Q   V   F   G   G   G
         GAG GCT GAT TAT TAC TGC CAG TCC TAT GAC AGC AGC CTG AAT GCT CAG GTA TTC GGA GGA GGG
               315                 324                 333                 342                 351                 360                 369

         T   R   L   T   V   L   G   Q   P   K   A   A   P   S   V   T   L   F   P   P   S
         ACC CGG CTG ACC GTC CTA GGT CAG CCC AAG GCT GCC CCC TCG GTC ACT CTG TTC CCG CCC TCC
               378                 387                 396                 405                 414                 423                 432

         S   E   E   L   Q   A   N   K   A   T   L   V   C   L   I   S   D   F   Y   P   G
         TCT GAG GAG CTT CAA GCC AAC AAG GCC ACA CTG GTG TGT CTC ATA AGT GAC TTC TAC CCG GGA
               441                 450                 459                 468                 477                 486                 495

         A   V   T   V   A   W   K   A   D   S   S   P   V   K   A   G   V   E   T   T   T
         GCC GTG ACA GTG GCC TGG AAG GCA GAT AGC AGC CCC GTC AAG GCG GGA GTG GAG ACC ACC ACA
               504                 513                 522                 531                 540                 549                 558

         P   S   K   Q   S   N   N   K   Y   A   A   S   S   Y   L   S   L   T   P   E   Q
         CCC TCC AAA CAA AGC AAC AAC AAG TAC GCG GCC AGC AGC TAC CTG AGC CTG ACG CCT GAG CAG
               567                 576                 585                 594                 603                 612                 621

         W   K   S   H   R   S   Y   S   C   Q   V   T   H   E   G   S   T   V   E   K   T
         TGG AAG TCC CAC AGA AGC TAC AGC TGC CAG GTC ACG CAT GAA GGG AGC ACC GTG GAG AAG ACA
               630                 639                 648                 657                 666                 675                 684

         V   A   P   T   E   C   S
         GTG GCC CCT ACA GAA TGT TCA TGA
               693                 702                 711
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FIG. 10a



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     882         891         900         909         918         927         936
H   N   A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S   V
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC
    945         954         963         972         981         990         999

L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   K
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA
   1008        1017        1026        1035        1044        1053        1062

A   L   P   A   P   I   E   K   T   I   S   K   A   K   G   Q   P   R   E   P   Q
GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG
   1071        1080        1089        1098        1107        1116        1125

V   Y   T   L   P   P   S   R   D   E   L   T   K   N   Q   V   S   L   T   C   L
GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG
.  1134        1143        1152        1161        1170        1179        1188

V   K   G   F   Y   P   S   D   I   A   V   E   W   E   S   N   G   Q   P   E   N
GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC
   1197        1206        1215        1224        1233        1242        1251

N   Y   K   T   T   P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L
AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC
   1260        1269        1278        1287        1296        1305        1314

T   V   D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E   A
ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT
   1323        1332        1341        1350        1359        1368        1377

L   H   N   H   Y   T   Q   K   S   L   S   L   S   P   G   K   .
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
   1386        1395        1404        1413        1422        1431
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FIG. 10b-2



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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10053

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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